1947-Pos Board B717
Identification and Function of Leash Forming Residues in the F_OF_1 ATP Synthase Molecular Motor using Single Molecule Measurements
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In single molecule rotation measurements, the transient dwells in the proteolipid ring rotation of *E. coli* F_oF_1-ATP Synthase were eliminated by either mutation of aE196L or cR50L to subunit a and subunit c, respectively, of the F_o motor. These data indicate that these residues are involved in the formation of the F_o motor leash that allows rotary motion of the c-ring to a limit of ~36° while engaged. These mutations decreased the rate of oxidative phosphorylation dependent growth to one half of that observed with wild type. The ability of ATPase dependent or NADH-dependent proton gradient formation across inverted membrane vesicles from *E. coli* was measured by ACMA quenching. Mutations to either residue aE196 or cR50 that altered the ability to form a salt bridge between these subunits were observed to increase the rate and extent of proton gradient formation induced by either ATP or NADH. However, none of these mutations decreased the rate of ATP hydrolysis activity of the F_oF_1 in the inverted membrane vesicles significantly. These results suggest that the ability to form the leash between subunits a and c biases rotation of the c-ring in the ATP synthesis direction.

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High-Throughput Single-Molecule Analysis of Nucleic Acid Enzyme Activity by Tethered Particle Motion
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Tethered Particle Motion (TPM) has the advantage to explore the conformational dynamics of DNA molecules, isolated or interacting with proteins, in the absence of external forces [1]. To take full benefit of this method, we developed a biochip that consists of microarrays of functional sites patterned by a soft lithography technique and thus enables the simultaneous analysis of hundreds of single DNA molecules by TPM.
Using our TPM-on-a-chip method we investigated the activity of the T7 gene exonuclease. This enzyme leads to the conversion of dsDNA to ssDNA and was classified according to previous bulk analyses as non processive in contrast to other exonucleases. Our results give eveidence for a processivity of the enzyme and revealed subtle characteristics of its mechanism which could not be unveiled by conventional approaches.
[1] Manghi M., Tardin C. et al. *Probing DNA conformational changes with high temporal resolution by Tethered Particle Motion.* Phys. Biol. 7: 046003 (2010).

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Detection of Polymorphic Transformations in Bacterial Flagellar Filaments using Solid Sate Nanopores
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Over the past 15 years nanopore based devices have emerged as promising tools for single molecule detection and analysis. These devices rely on detecting changes in current between two electrolytic half cells separated by a nanopore bearing membrane, due to the displacement of the electrolyte within the nanopore by an analyte being driven electrophoretically across it. More recently, such devices have been used to characterize protein folding/unfolding mechanisms and binding kinetics by relating the differences in the amplitude and duration of the current blockade to expected biophysical changes in the analytes' structure. However given the complex and dynamic nature of most proteins' structure such approaches are largely empirical and often difficult to corroborate through simulations. In order to elucidate how structural variations in a protein relate to changes in the observed current blockades we present a systematic analysis of the structural variations in protein filaments using solid state nanopores.
Bacterial flagellar filaments are chosen as the analyte in the presented work since these proteinaceous organelles acquire simple helical structures whose pitches change reversibly in response to changes in the fluidic environment such as its pH and salinity. Using a 250 nm diameter pore, we parametrically vary the pH and salinity of the electrolyte and detect corresponding polymorphic transformations in the flagellar filaments. Further, given the simplicity of these structures, such transformations are easy to model mathematically and we show that the changes in the nature of the current blockade closely agree with those predicted by our model. Moreover, given the sensitivity of the flagellar filaments to ambient conditions, our setup could also be used as a nanoscale fluidic sensor capable of detecting minute changes in the fluidic environment in and around the nanopore.

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Stochastic Properties of Transcription Factor Expression Revealed by Single-Molecule Noise Analysis
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Transcription factors (TFs) play important roles in gene regulation. Monitoring TF production in real time will help to elucidate how the stochasticity in the expression TFs influences the expression of their target genes. However, many TFs are expressed at low levels and are thus difficult to detect. Further, labeling TFs with fluorescent proteins can potentially disrupt their regulatory functions. Here, we developed a novel strategy, Co-Translational Activation by Cleavage (CoTrAC), to monitor the stochastic expression of a TF, λ repressor CI, in live *E. coli* cells at the single-molecule level. CI becomes fully functional upon co-translational cleavage from a membrane-targeted reporter, which can be counted individually. Using this strategy, we monitored the production of CI in real time in the regulatory context of the λ phage genetic switch. We developed a robust noise analysis to decompose the total noise in CI production into intrinsic, extrinsic and correlation sources. We show that intrinsic noise likely arises from both transcriptional and translational bursting. Extrinsic noise accounts for most of the total noise on the time scale of one cell cycle. CI enhances transcription primarily by increasing bursting frequency. Furthermore, negative autoregulation increases intrinsic noise, but counteracts extrinsic noise and ensures that molecular memory diminishes on a time scale shorter than one cell cycle. The experimental and theoretical approaches presented here offer a unique opportunity to investigate noise in gene regulatory networks.

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3D Tracking of Cellular Nanoscale Dynamics with Sub-Millisecond Temporal Resolution
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The study of highly dynamic cellular processes is leveraged by microscopic techniques with high temporal resolution. Especially for diffusive processes, where particle displacements can exceed 100 nm/ms, sub-millisecond temporal resolution is often required in addition to precise three-dimensional localization.
We have developed a novel particle tracking microscope capable of observing fast 3D trajectories of fluorescently tagged intracellular objects with 300 μs temporal resolution [1]. Combining scanning-free biplane detection [2] with optimized beam steering, we were able to demonstrate 3D tracking of single fluorescent particles at speeds of up to 150 nm/ms over several seconds and large volumes. Maximum detection efficiency and minimal laser irradiation are guaranteed by focused excitation of only the particle of interest and by exploiting the high quantum efficiency of an EM-CCD camera. Limiting detection to a small subarea of the CCD chip improves speed and reduces background fluorescence. Combined with the absence of sample movement, these features ensure high live-sample compatibility.
Here, we present recent improvements of the setup using fast adaptive optics for axial scanning. This enhances response times and eliminates mechanical coupling of moving components to the sample. We also show data from recent live-cell experiments. We have tracked actin-driven motion of GFP-labeled HIV-like particles on the cytoplasmic membrane. This "surfing" is involved in retroviral cell-to-cell spread [3] and precedes endocytosis. Fast 3D particle tracking has the potential to contribute to the understanding of the underlying mechanism.
[1] M. F. Juette and J. Bewersdorf, Nano Lett., 10(11), 4657-4663 (2010).
[2] M. F. Juette et al., Nat. Methods, 5(6), 527-529 (2008).
[3] W. Mothes et al., J. Virol., 84(17), 8360-8368 (2010).

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Effects of Viscosity on Tethered Particle Motion (TPM) Experiments
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Tethered Particle Motion (TPM) experiments are used to estimate the extension of a DNA molecule, attached at one end to a glass surface, and at the other